PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 37/54, 39/394 — A1

(11) International Publication Number: WO 94/21284

(43) International Publication Date: 29 September 1994 (29.09.94)

(21) International Application Number: PCT/AU94/00121

(22) International Filing Date: 15 March 1994 (15.03.94)

(30) Priority Data:
PL 7821 15 March 1993 (15.03.93) AU

(71) Applicant *(for all designated States except US)*: PHARMA PACIFIC PTY. LTD. [AU/AU]; (ACN 007 426 379), 103-105 Pipe Road, Laverton, VIC 3026 (AU).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: CHANDLER, David, Spencer [AU/AU]; "Trenwell", Brassey Court, Mickleham, VIC 3064 (AU). REED, Benjamin, John [AU/AU]; 19 Frederick Street, Ferntree Gully, VIC 3156 (AU).

(74) Agent: SANTER, Vivien; Griffith Hack & Co., 509 St. Kilda Road, Melbourne, VIC 3004 (AU).

(81) Designated States: AU, CA, CN, JP, NZ, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: THERAPEUTIC FORMULATION AND METHOD

(57) Abstract

The invention provides a method of treatment or prevention of gastrointestinal disease in an animal, comprising the step of administering to an animal in need of such treatment an effective amount of an antibody which has been pretreated with a proteolytic enzyme, or of a proteolytic enzyme together with an effective amount of an antibody, optionally in conjunction with a probiotic organism, wherein the antibody has specificity against an organism capable of causing gastrointestinal disease. Preferably the antibody is derived from colostrum. The invention also provides compositions for use in the method.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

THERAPEUTIC FORMULATION AND METHOD

This invention relates to a method and composition for treatment or prevention of gastrointestinal disease, particularly in neonatal mammals.

Background and Prior Art

Gastrointestinal disease is a significant cause of morbidity and mortality in humans and in domestic animals, particularly in the first few weeks of life. A high proportion of hospital admissions of babies results from gastrointestinal infection, which leads to rapid dehydration, and may prove fatal. Among domestic animals, particularly in intensive rearing situations, gastrointestinal infection spreads extremely rapidly, and results in failure to thrive, often leading to death. The effects of these conditions are particularly devastating in the production of pigs and poultry.

While in human patients the treatment largely depends on oral or intravenous rehydration therapy, in the farm situation efforts to contain gastrointestinal infection have largely relied upon feeding of large amounts of antibiotics in either feed or water. This is very costly and suffers from the disadvantage that resistance of the causative organisms to the antibiotics is likely to arise, and to spread to other, possibly more dangerous organisms. Hitherto vaccines have proved unreliable.

Since the causative organisms of gastroenteritis have receptors on the cell surface for binding to the intestinal mucosa, oral administration of enzymes such as papain or bromelain has been proposed to try to prevent infection. A protease preparation has been marketed under the trade name "DETACH" by Ciba-Geigy.

In most domestic animals, maternal antibodies are transferred to the progeny in colostrum. In situations where young animals are intensively reared, and are not kept with their mothers, various artificial colostrum products have been used in an attempt to provide passive

immunity. For example a product named "Gamma Sow" formerly produced by the Victorian Department of Agriculture and a product named "Revive" manufactured by Bayer, both utilise immune serum from slaughtered sows. Other products, available overseas, use immunoglobulins obtained from colostrum, milk or whey. However, most of these products are extremely expensive or are not available in Australia.

In the case of rotavirus diarrhoea, it is known that the most important protective factor is the presence of specific antibody in the lumen of the small intestine. Protection against rotavirus diarrhoea can be achieved by oral administration of IgG, whether the IgG is homologous or heterologous (Snodgrass D.R. *et al*, Infect. Immun., 1977 16 268-270; Barnes, G.L. *et al*, Lancet 1982 1 1371-1373). However, it appeared from these references that it was necessary that the IgG should be purified, or should be present in colostrum rather than in milk. It was also shown that cows that had been immunised with inactive bovine rotavirus conferred passive antibody to their calves via colostrum (Mebus, C.A. *et al*, J. Am. Vet. Med. Assoc., 1973 163 880-883). It was subsequently shown that oral administration of bovine colostrum from immunized cows to human infants was effective in protection against rotavirus diarrhoea, (Hilpert H. *et al*, J. Infect. Dis., 1987 156 158-166; Davidson G.P. *et al*, Lancet 23 September 1989 709-712).

Intact colostral antibody has been found efficacious in treatment of *Helicobacter pylori* infections, which may be associated with gastritis and peptic ulcer disease. *Helicobacter pylori* was formerly known as *Campylobacter pylori*. This method is the subject of Australian Patent Application Number 80207/91 by Abbott Laboratories, entitled "Method for the treatment of gastric disease", the entire disclosure of which is herein incorporated by reference. Efficacy in this instance was obtained by regular ingestion of intact colostral whey antibody. This specification describes in detail methods

for immunisation with *Helicobacter pylori*, and methods for isolation and concentration of specific antibodies from mammary secretions, including milk and colostral whey, of animals immunised with *Helicobacter pylori*, and in particular bovine colostrial whey.

Methods for production of immunoglobulins with specificity against various organisms from lactating mammals are also disclosed in U.S. Patents No. 3128230 and No. 4051231. Australian Patent Application No. 644468 (82527/91) discloses a process for preparation of a spray-dried colostrum product which can be applied to immune or hyperimmune colostrum, which is stated to be useful in the treatment or prevention of rotavirus infection in infants.

Immunoglobulins consist of Y-shaped molecules which may be associated into multimers. These basic units are readily cleaved by proteolytic attack into the Fab (antigen binding) portion and the Fc (constant) fragment. The Fab moiety contains the complementarity-determining regions which act as the specific antigen binding portion. The Fc region is involved in binding to certain host cell surfaces, usually after conformational changes in the molecule which occur following antigen binding, and in the binding of complement. Fc binding initiates a number of downstream immunological reactions which are ultimately directed towards removal of the antigen from the host body.

Many organisms, including bacteria, mycoplasma, viruses and protozoa have evolved receptors which bind free Fc regions. The presence of microbial Fc receptors on the cell surface is correlated with pathogenicity and virulence, and also with suppression of the host immune response (Widders, P.R.; Bacterial Immunoglobulin-Binding Proteins, Vol. 1 (Academic Press) 1990 Pages 375-395). The Fc receptors may remain fixed on the microbial surface, or may be sloughed to become "soluble" Fc receptors. Strong presumptive evidence indicates that microbial Fc receptors favour persistence of organisms in mammalian hosts via a variety of mechanisms, including reduction of opsonisation

and phagocytosis, reduction of complement activity, and possibly reduction of antibody-dependent cell-mediated cytotoxicity and metagenesis.

The Fc portion of IgM, which is a pentamer of the Y-shaped basic units, has been demonstrated to enhance clearance of *E. coli* strain 055 in new born, pre-colostral piglets (Zikan, J. and Miler, I., Immunochemistry 1975 12 813-815). Although the presence of Fc receptors was not detected on these bacteria, Fab fragments obtained by pepsin digestion of IgM retained the complement-associated bactericidal activity of the parent molecule, while the Fc fragment retained the ability of the parent molecule to clear *E. coli* by opsonisation.

Orally-administered protease alone has been shown to influence the potential for microbial colonisation of the small intestine by degrading receptors for microbial adhesion and microbial toxin (Chandler, D.S., Ph.D. Thesis, La Trobe University 1986; Mynott *et al*, Infection and Immunity, 1991 59 3708-3714).

Hyperimmune colostral antibodies directed against organisms causing gastrointestinal disease have been recognised to be effective in disease control (Tackett *et al.*, New England J. Med. 1988 318 1240-1243; Hilpert *et al*, J. Infect. Dis., 1987 156-158; Ebina *et al*, Med. Microbiol. Immunol., 1985 174-177; Davidson *et al*, Lancet, 1989, 23 September 709-712).

Although, as stated above, it is well known that limited digestion of immunoglobulin molecules with proteolytic enzymes such as pepsin and papain cleaves the immunoglobulin to form Fc and either Fab or $Fab(_2)$ fragments, this is effected by limited digestion only, and must take place under controlled conditions. Unless the conditions are carefully controlled, some proteases will completely break down the antibody, and destroy its activity.

We have now surprisingly found that an improved response may be obtained by combining administration of

protease-treated antibody in order to prevent or alleviate gastrointestinal disease. Administration of protease-treated antibody, or protease together with antibody, enables each component to exert its separate effects, and also ensures that at least a portion of the specific antibody is affected. This is particularly beneficial in neonates, where gastric and intestinal proteolytic activity is low because of developmental immaturity, (Moughan P.J. *et al*, In. Nutritional Triggers for Health and in Disease; Simopoulos A.P. (ED) Worlds Rev. Nutr. Diet. Basel, Karger, 67 40-113) and in adults, where gastric stasis may interfere with the maintenance of integrity of ingested antibody.

In the neonate, gastric and pancreatic secretory activity is not fully developed, and in addition the gastric pH is relatively high; there is therefore no trigger for pancreatic enzyme release, and the pH is too high for pepsin to be active. Furthermore, during development, the enzyme chymosin appears before pepsin; although chymosin can clot milk, it is unable to cleave antibody. It would therefore be expected that in the neonate colostral antibody would pass through the stomach without being broken down. The late appearance of pepsin, commencing about one week after birth, has conventionally be thought to be beneficial because antibody is not broken down in the stomach (Foltman, B., 1975 In. Proc. 3rd Int. Semin. Dig. Physiol. Pig., Just, Jorgensen, Fernandez (Eds) 120-123 National Institute of Animal Science, Copenhagen). It is therefore particularly surprising that we have found that administration of colostral antibody which has been treated with proteolytic enzymes has a beneficial effect in neonatal piglets.

Because of the apparent favouring of development of Gram positive flora (lactobacili, streptococci,or both) in the gastrointestinal tract (GIT) following treatment with pepsin-digested antibody, the present invention proposes that an exogenous culture of these organisms, if

administered concurrently with the protease-treated antibody, would have an improved chance of colonisation. Cultures of lactobacilli and streptococci are currently used commercially, with limited success, to control diarrhoeal diseases in piglets and other species. These cultures are called probiotics. The main problem with the therapeutic function of probiotics is the difficulty in establishing these strains in the GIT (Cain, C.,1988. Observations of indigenous and non-indigenous lactic acid bacteria as potential probiotic organisms in pigs. Masters Thesis, School of Agriculture, La Trobe University).

The present invention provides a means of improving the likelihood of improving the colonisation of the intestinal tract by probiotic strains, thereby extending the period of disease protection offered by the oral administration of antibody. It is proposed that the use of pepsin-digested antibody in neonates, or undigested antibody in older individuals together with probiotics, may be used to treat both gastric and intestinal infections. The combination of antibody and an appropriate probiotic would greatly reduce the requirement for continuous antibody therapy.

Summary of the Invention

The invention therefore provides in one aspect a method of treatment or prevention of gastrointestinal disease in an animal, comprising the step of administration to a mammal in need of such treatment of an effective amount of an antibody which has been pretreated with an appropriate proteolytic enzyme, or of a proteolytic enzyme together with an effective amount of an antibody.

The invention is applicable to the treatment of a wide variety of animals, including pigs, cattle, sheep, horses, poultry and humans. It is particularly suitable for the treatment of neonatal animals and humans. The causative organisms of the gastrointestinal disease which may be treated or prevented include, but are not limited

to, *Helicobacter pylori*, *Escherichia coli*, and rotavirus.

The antibody suitable for use in the invention does not have to be purified, and may be derived from immune serum or colostrum, or from yolks of eggs of immunized poultry, or may be a monoclonal antibody or a bioengineered antibody. The only requirement is for antibody specificity against the pathogenic agent. Colostrum from immunised dairy animals, such as cows, sheep or goats, is especially convenient for use in the invention. The antibody may be IgG, IgA or IgM, but is preferably IgG_1, and is most preferably bovine IgG_1. If the antibody is derived from egg yolk it is preferably Igγ.

Proteolytic enzymes which are suitable for use in the invention include, but are not limited to, pepsin, papain, bromelain, fungal proteases, and trypsin. Pepsin is preferred, because it is easy to control the digestion (cleavage) reaction, and because it is cheap and robust. The concentration of the proteolytic enzyme should not be so high, or the digestion so prolonged, that the antibody is totally degraded; the person skilled in the art will be able to determine a suitable concentration by normal trial and error experimentation.

In an alternative aspect, the invention provides a method of treatment or prevention of gastrointestinal disease in an animal, comprising the step of administering to an animal in need of such treatment an effective amount of an antibody which has been pretreated with a proteolytic enzyme, or of a proteolytic enzyme together with an effective amount of an antibody, in conjunction with a probiotic organism. The probiotic organism is suitably an organism indigenous to the species of animal to be treated, although it may be a non-indigenous member of the mucosal flora of healthy individuals of that species. Preferably the probiotic organism is a *Lactobacillus* or *Streptococcus*. A mixture of two or more probiotic organisms may be used.

In either aspect, the method of the invention may be used in conjunction with other treatments, such as

antibiotic treatment.

Optionally further protease may be administered separately, in order to influence mucosal properties which favour chemical interactions between a pathogenic organism and the host.

For the purposes of the present specification, the terms "proteolytic enzyme" and "protease" are to be taken to be synonymous.

While the invention is specifically described with reference to gastrointestinal disease, it will be clearly understood that the invention is applicable to the treatment or prevention of disease at other sites which is caused by organisms from the gastrointestinal tract of the animal suffering the disease.

Depending on the type and activity of the protease which is used for immunoglobulin pretreatment, the antibody and the protease may have to be delivered separately.

The methods of the invention are suitable for treatment of immunocompromised patients or patients particularly prone to infection, such as patients with AIDS-related complex or AIDS, or patients suffering rom extensive burns or scalds, or for the treatment of patients suffering from gastrointestinal malabsorption syndromes.

The methods of the invention are also suitable for treatment of patients receiving H_2-receptor antagonists such as Tagamet, which inhibit acid secretion in the stomach, and have diarrhoea as a frequent side-effect.

In a second aspect, the invention provides a composition for treatment or prevention of gastrointestinal disease in an animal, comprising either

a) an effective amount of an antibody which has been pretreated with a proteolytic enzyme or

b) a proteolytic enzyme together with an effective amount of antibody,

and optionally a probiotic organism, together with a pharmaceutically-acceptable carrier.

In a preferred embodiment for administration to adult subjects, the formulation provides a two-part liquid format, in which the protease is enteric coated or buffered, and is suspended in a buffered liquid excipient either separately or together with the antibody.

In an alternative embodiment, the formulation may comprise a multilayer tablet, optionally enteric coated, in which the enzyme forms the innermost layer and antibody forms an outer layer, preferably isolated from the enzyme. Conventional fillers, granulating agents, and excipients may be present. Variations will be obvious to the person skilled in the art.

The invention also provides formulations for use in the aforesaid method. Individual formulations will depend upon the antibody and protease type, and can be devised using known formulation principles and normal trial-and-error experimentation.

Detailed Description of the Invention

The invention will now be illustrated by way of reference only to the following non-limiting examples:

Example 1 Use Of Pepsin Digested Antibody To Control Diarrhoeal Disease In Piglets

This experiment was to investigate whether passive immune protection for piglets during challenge with pathogenic *E. coli* was best achieved using intact antibody contained in a high energy colostrum replacer specially formulated for use in piglets, as intact antibody purified from the same batch of colostrum, or as a peptic digest of the purified antibody preparation.

MATERIALS AND METHODS

Antibody Treatments

These consisted of six twenty ml doses given to piglets at approximately 6h intervals. Piglets in the colostrum replacer group received ReSus (Nufarm Animal

Health Pty Ltd). ReSus is a commercial colostrum replacer containing hyperimmune bovine colostrum from cows immunised against *E. coli* of types which infect piglets, using a polyvalent whole cell and pilus vaccine. Piglets in the second treatment group received bovine colostral antibody from the same bulk batch of colostrum, but in this case the antibody had been removed from the base colostrum by fat removal and acid casein precipitation. The antibody was then further purified by $(NH_4)_2SO_4$ precipitation and exhaustive dialysis against distilled water, until no precipitate was evident when the antibody containing solution was added to a $BaCl_2$ solution. Peptic digestion of the antibody for use with piglets in the third treatment group was conducted at 37°C overnight, using commercial pepsin in the ratio of one part pepsin to fifty parts antibody (Fang, W.D. and Mukkur, T.K.S., Biochem. J., 1976 155 25). All antibody-containing treatments were adjusted prior to use to have the same titre of blocking activity (equivalent to that found in ReSus) when tested in an ELISA blocking assay. This assay consisted of K88$^+$ *E. coli* on the solid phase, followed by test antibody, anti-K88 conjugate and enzyme substrate. A fourth (control) group of piglets was given the same volume of commercial milk replacer containing no anti-K88 antibody, according to the same regimen as piglets given the antibiotic treatments. Treatments were given by oro-gastric tube. Commercial milk replacer, at manufacturer's recommended quantity for neonatal piglets, and bacterial challenge doses were also given by oro-gastric tube.

Piglet Management

Piglets born to three sows were taken at birth, before they had a chance to suckle. The piglets were immediately weighed, ranked by weight and randomly distributed into four weight-matched treatment groups. Piglets were identified individually and by treatment group using individually numbered coloured eartags. They were

then allocated randomly to heated cages in approximately weight-matched pairs. At about two hours after birth the piglets were given their first treatment dose, followed thirty minutes later by a bacterial challenge dose, consisting of 10^{10} haemolytic K88$^+$ *E. coli*, strain WG, O149;K91;K88ac;H10, (Tzipori *et al*, Aust. Vet. J., 1980 56 274). A second similar challenge dose was given 24h later. All piglets were killed at 48h after birth by barbiturate overdose.

Microbiological Assessments

Immediately after death intestinal scrapings were taken from the stomach and from three sites in the small intestine. The stomach was sampled half way around the greater curvature, whilst the small intestine was sampled 200mm from either end and half way between. These sites were designated sites 1 (duodenal end), 3 and 2, respectively. Scrapings from 1cm^2 of mucosa at each site were suspended in sterile phosphate buffered saline (pH 7.2, 0.1M). Bacterial counts were then performed according to the method of Miles and Misra (1932) using Sheep Blood and MacConkey Agars incubated aerobically overnight at 37°C, and Rogosa and Trypticase Soya Agars (TSA); incubated aerobically and anaerobically for 48h at 37°C. Counts were made of haemolytic large colonies on the blood agar (some of which were confirmed by slide agglutination to be the challenge strain), and of small colonies (assumed to be streptococci). Coliform counts (both lactose fermenting and non-fermenting) were made on MacConkey Agar, and lactobacillus from the Rogosa Agar. The TSA was used to assess total bacterial count. Rogosa and TSA counts were similar when incubated aerobically or anaerobically. Lactobacilli numbers were estimated from anaerobically incubated plates. Total counts were estimated from aerobically incubated plates.

Statistical Analysis

Log transformed bacterial counts were found to have homogeneity of variance for the treatment groups. They were therefore analysed by Analysis of Variance and by spatial analysis(2D, NSW Dept of Agriculture).

RESULTS AND DISCUSSION

Analyses of the bacterial counts are illustrated in Tables 1-3. The results illustrate that the performance of the control group piglets was worse in terms of higher pathogen indicators (haemolytic or coliform counts) and lower counts of "desirable" populations of lactobacilli or streptococci, at all sites. This is as expected for piglets deprived of colostral protection. Generally bacterial counts were higher in the stomach and lower small intestine, although counts of the pathogenic strain were also high in the upper small intestines of the control group piglets. The influence of higher intestinal pathogen counts in the control group piglets was also reflected in poorer condition and faecal score values recorded for these piglets (data not shown). Streptococci and lactobacilli are bacterial populations of the GIT that are generally associated with good health. Counts of these bacteria were higher in all piglets receiving antibody treatments. Table 1 indicates that the purified antibody counts were not significantly improved over commercial colostrum replacer, although there were trends within the results for both lower pathogen counts and higher "desirable flora" counts overall within the GIT, particularly in piglets receiving pepsin-digested antibody. Analysis of these counts as a desirable:undesirable population ratio (Table 2) indicated an improved ratio for the pepsin-digested antibody treatment over the ratio obtained with the undigested (purified) antibody of 28.2% for the haemolytic: lactobacilli ratio and 21.2% for the haemolytic: streptococci ratio. Table 3 illustrates the high variability between the site: site and pig: pig counts

generally, and the difficulty in demonstrating significant treatment effects with small numbers of piglets. The trend of lower pathogen counts to non-pathogen counts in piglets receiving pepsin digested antibody was still a consistent pattern, however, being evident in most GIT sites (12 of 16). In Tables 2 and 3, the difference between treated and control for each treatment is significant if it is greater than the LSD 5% value. These results support the hypothesis that pepsin-digested antibody treatment better favours development of a Gram positive populations on the mucosal surface, whilst maintaining or improving suppression of the pathogen population. The observations of this trial are consistent with the hypothesis that free Fc fragments, which predominate in the digested antibody preparation, may favour development of a Gram positive (Fc receptor-possessing) mucosal flora, whilst suppression of a pathogenic population is still effectively achieved by the specific antigen binding properties of the Fab antibody fragment.

Table 1

Means of log transformed bacterial counts over all GIT sites

TREATMENT	Haemolytic (SBA)	Lactobacillus (Rogosa)	Coliform (Mac)	Streptococci (SBA)	Total
Control	5.82^{a*}	4.70^{a}	6.54^{a}	3.13^{a}	7.22
ReSus	5.02^{ab}	5.52^{ab}	5.48^{ab}	4.61^{b}	6.81
Undigested Antibody	3.96^{b}	5.32^{ab}	5.24^{ab}	4.36^{b}	6.24
Pepsin-Digested	3.39^{b}	6.01^{b}	4.83^{b}	4.97^{b}	6.29

* Comparable means with similar superscripts are not significantly different at the $p<0.05$ level,

Values marked a and b are not significantly different from any other values marked a or b respectively, and those marked a or b are not significantly different from those marked ab.

Table 2

Means of log transformed bacterial count ratios over all GIT sites.

TREATMENT	Haemolytic/Lactobacillus	Haemolytic/Streptococci
Control	1.51^{a*}	2.4^{a}
ReSus	0.93^{ab}	1.4^{ab}
Undigested Antibody	0.78^{b}	1.1^{ab}
Pepsin-Digested Antibody	0.56^{b}	0.8^{ab}
LSD 5%*	0.68	1.34

* Comparable means with similar superscripts are not significantly different at the $p<0.05$ level.

* LSD 5% is the least significant difference at the 5% confidence level.

Table 3 Actual means of log transformed bacterial counts, and p values for treatment effects determined by analysis of variance at each site

a MEANS FOR HAEMOLYTIC COUNTS

TREATMENT	Stomach	Site 1	Site 2	Site 3
Control	5.411	6.157	4.831	6.523
ReSus	4.515	4.410	4.147	6.206
Undigested Antibody	3.581	3.037	3.574	4.927
Pepsin-Digested Antibody	3.320	2.466	3.730	3.710
p value	0.310	0.070	0.860	0.270
LSD 5%		3.510		

b MEANS FOR LACTOBACILLUS COUNTS

TREATMENT	Stomach	Site 1	Site 2	Site 3
Control	5.615	4.825	3.676	3.886
ReSus	6.212	5.349	4.728	5.578
Undigested Antibody	5.662	5.268	5.142	5.258
Pepsin-Digested Antibody	7.250	5.544	4.633	6.184
p value	0.040	0.880	0.310	0.080
LSD 5%				1.760

c MEANS FOR COLIFORM COUNTS

TREATMENT	Stomach	Site 1	Site 2	Site 3
Control	6.089	7.201	5.341	7.202
ReSus	5.086	4.766	4.863	6.441
Undigested Antibody	4.277	4.712	4.879	6.569
Pepsin-Digested Antibody	4.960	3.679	3.992	5.518
p value	0.400	0.080	0.790	0.600
LSD 5%		2.690		

Table 3 (continued)

d MEANS FOR STREPTOCOCCAL COUNTS

TREATMENT	Stomach	Site 1	Site 2	Site 3
Control	3.670	2.289	2.520	3.583
ReSus	5.599	3.109	4.827	4.472
Undigested Antibody	4.733	3.641	4.331	4.497
Pepsin-Digested Antibody	6.548	3.980	5.302	3.849
p value	0.040	0.500	0.260	0.830
LSD 5%	1.920			

e MEANS FOR TOTAL COUNTS

TREATMENT	Stomach	Site 1	Site 2	Site 3
Control	7.013	7.908	6.141	7.652
ReSus	6.852	6.029	6.959	7.114
Undigested Antibody	5.787	5.705	5.614	7.358
Pepsin-Digested Antibody	7.179	6.107	5.780	5.812
p value	0.390	0.310	0.720	0.310

EXAMPLE 2 FORMULATION AS TWO TYPES OF MICROCAPSULES IN A CAPSULE SHELL

A formulation according to the invention comprises a capsule shell, containing two types of microcapsules:

a) a protease selected from bromelain, pepsin or papain, together with binder agents, present as cores about 750 microns in diameter, and coated with an enteric coating agent;

b) antibody such as bovine IgG, in a preferably non-protein base microencapsulated with bovine colostrum; the microcapsules should have a core size of not more than 250 microns.

Suitable agents include starch, carboxymethyl cellulose, povidone, lactose and dextrose. Suitable enteric coating agents include cellulose acetate phthalate, with a suitable film softening agent such as triacetin or glycerine. The microspheres are formed using a standard device such as a Rota-processor (Acromatic A.G.) and then the protease microspheres may be coated using an Ultra Coater (Acromatic A.G.). Similarly, antibody microspheres may be formed in a Rota-processor and the coating of bovine colostrum may be applied by top spray coating.

Microcapsules prepared as in this example may be formulated as a tablet in a starch base.

EXAMPLE 3 **TABLET FORMULATIONS ESPECIALLY SUITABLE FOR ADULT SUBJECTS**

Three alternative tablet formations are as follows:

a) a core of bromelain, together with starch filler is coated with cellulose acetate phthalate, then with a layer of antibody together with colostrum and egg albumin.

b) a core of bromelain, antibody, starch and egg albumin is coated with a layer of colostrum, and then with a layer of cellulose acetate phthalate.

c) a core of bromelain, antibody, starch and egg albumin is coated with cellulose acetate phthalate and then with an outer layer of colostrum.

EXAMPLE 4

In the formulations according to Examples 2 and 3(b) and (c), the bromelain and antibody may be replaced by antibody which has been pre-treated with a proteolytic enzyme.

It will be clearly understood that the invention in its general aspects is not limited to the specific details referred to hereinabove.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of treatment or prevention of gastrointestinal disease in an animal, comprising the step of administration to an animal in need of such treatment of an effective amount of an antibody which has been pretreated with a proteolytic enzyme, or of a proteolytic enzyme together with an effective amount of an antibody, wherein said antibody has specificity against an organism capable of causing gastrointestinal disease.

2. A method of treatment or prevention of gastrointestinal disease in an animal, comprising the step of administering to an animal in need of such treatment an effective amount of an antibody which has been pretreated with a proteolytic enzyme, or of a proteolytic enzyme together with an effective amount of an antibody, in conjunction with a probiotic organism, wherein said antibody has specificity against an organism capable of causing gastrointestinal disease.

3. A method according to Claim 1 or Claim 2 in which the gastrointestinal disease is gastroenteritis or diarrhoea.

4. A method according to any one of Claims 1 to 3 in which the gastrointestinal disease is caused by an organism selected from the group consisting *Escherichia coli*, *Helicobacter pylori* and rotavirus.

5. A method according to any one of the preceding claims in which the antibody is derived from a source selected from the group consisting of immune serum, immune colostrum, a monoclonal antibody, and a bioengineered antibody.

6. A method according to Claim 5 in which the antibody is derived from colostrum of an immunized diary animal.

7. A method according to Claim 6 in which the antibody is bovine IgG_1.

8. A method according to any one of the preceding claims in which the proteolytic enzyme is selected from the

group consisting of pepsin, papain, bromelain, fungal proteases and trypsin.

9. A method according to Claim 2 in which the probiotic organism is a *Lactobacillus* or a *Streptococcus*.

10. A method according to any one of the preceding claims, in which the animal is a neonatal human, piglet, calf, foal, lamb goat or bird, including poultry, and the gastrointestinal disease is a diarrhoeal disease.

11. A method according to any one of Claims 1 to 9 wherein the mammal is a human selected from the group consisting of immunocompromised patients, patients particularly prone to infection, patients suffering from gastrointestinal malabsorption syndrome, patients undergoing treatment with H_2-receptor antagonists patients suffering from antibiotic-associated diarrhoea, and patients suffering from travellers' diarrhoea.

12. A composition for treatment or prevention of gastrointestinal disease in a mammal, comprising either,

a) an effective amount of an antibody which has been pretreated with a proteolytic enzyme, or

b) a proteolytic enzyme together with an effective amount of antibody, and optionally

c) a probiotic organism,

together with a pharmaceutically-acceptable carrier, wherein said antibody has specificity against an organism capable of causing gastrointestinal disease.

13. A composition according to Claim 12 comprising a two-part liquid format, in which the proteolytic enzyme is enteric coated or buffered, and is suspended in a buffered liquid excipient either separately or together with the antibody.

14. A composition according to Claim 12, comprising a multilayer tablet, optionally enteric coated, in which the enzyme forms the innermost layer and the antibody forms an outer layer.

15. A composition according to Claim 14 wherein the enzyme layer is isolated from the antibody layer.

16. A composition according to Claim 12 adapted for addition to an animal feed preparation.

17. A composition according to Claim 12 which is adapted for addition to an infant food composition.

18. A composition according to Claim 12 which is adapted for addition to poultry feed or water.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 94/00121

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl.[5] A61K 37/54 39/394

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC A61K 37/54 39/394

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)
DERWENT: Ig: and proteolytic enzyme:
JAPIO: Ig: and proteolytic enzyme:

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	Patent Abstracts of Japan JP,A,2-1553 (FUJI YAKUHIN KOGYO KK) 5 January 1990 (05.01.90) abstract	1-18
A	Chemical Abstracts Volume 107, No 15, issued 12 October 1987 (12.10.87) Columbus, Ohio, USA, Shimazaki et al., Susceptibility oif bovine colostral and serum IgG to proteolytic enzymes as analyzed via gel filtration chromatographic behaviour., page 554, column 1, the abstract No. 132267e Nippon Chikusan Gakkaiho 1987, 58(4), 324-32 (Japan) abstract	1-18

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
28 June 1994 (28.06.94)

Date of mailing of the international search report
6 July 1994 (06.07.94)

Name and mailing address of the ISA/AU

AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION
PO BOX 200
WODEN ACT 2606
AUSTRALIA

Facsimile No. 06 2853929

Authorized officer

HELLENE FLAME

Telephone No. (06) 2832253

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 94/00121

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	**Citation of document, with indication, where appropriate of the relevant passages**	**Relevant to Claim No.**
A	EP 0247998-A (IMMUNO Aktiengesellschaft) 21 May 1987 (21.05.87) whole document	1-18
A	WO 9322429-A1 (SHRINERS HOSPITALS FOR CRIPPLED CHILDREN) 29 April 1992 (29.04.92) whole document.	1-18

INTERNATIONAL SEARCH REPORT
Information on patent family meml

International application No.
PCT/AU 94/00121

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member					
EP,A,247998	DE	3786838	DK	2722/87	US	4814277
WO,A,9322429						
JP,A,2-1553	JP	3207618	JP	4297794	JP	5032878
	JP	545393	DE	4000817	EP	437738
	FI	910153	JP	4297794	US	5111607
	JP	5032878	JP	5249853	JP	545393
	JP	5288773	JP	5195086	JP	545393
	JP	5177313	JP	4316061		

END OF ANNEX